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ANTI-AGING EFFECTS OF TOPIC TREATMENT AND DIETARY SUPPLEMENTS OF LYCOPENE ON WOMEN

Cioffi Edvige[1], Pagano Imma[1,2]

[1]Post Graduate Universitary Course in "Diete e Terapie Nutrizionali Chetogeniche: Integratori e Nutraceutici (NutriKeto)" - Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II – Fisciano (SA), Italy

[1,2]Nutriketo Lab, A.O.R.N. 'San Giuseppe Moscati', Contrada Amoretta, Avellino, Italy

Email address: edvigecioffi@hotmail.com, nutriketo@unisa.it

Abstract

The plant kingdom is rich in bioactive compounds with antioxidant activity; one of the most important is lycopene. Lycopene is a carotenoid responsible for the red colour of tomatoes. Many studies have shown that lycopene has numerous biological properties useful for human health. At skin level it seems to protect from damage caused by UV and photoaging. In the present work, the anti-aging property of lycopene is proposed, analysing two studies about the administration of lycopene topically and systemically. The first study reported a tomato powder formulated as lotion while in the second one there is a dietary supplement containing lycopene plus other natural compounds. Both treatments are able to reduce wrinkles.

Keywords: *lycopene, tomato, anti-aging, wrinkles.*

Introduction

History and property of the tomato

The tomato is known in botany as *Solanum lycopersicum* (lycopersicum is interpreted literally from Latin in the 1753 book, Species Plantarum, as "*wolfpeach*", where wolf comes from lyco and peach is from persicum). It is an annual herbaceous plant belonging to the family of *Solanaceae*, whose red fruits are the emblem of Mediterranean diet. The word "tomato" comes from the Spanish "*tomate*", which in turn comes from the Nahuatl word "*tomatl*", meaning "*the swelling fruit*". In Italy the primitive term was "*mela aurea*" and this name over the years became the most immediate translation: pomod'oro or more simply pomodoro, due to the initial golden colour of the vegetable. The tomato owes its appearance in Europe to the Spanish conquistadores. In 1600 the tomato began to be cultivated for food purposes and finding the most favourable weather conditions, it changed its golden yellow colour to the current ruby red. The tomatoes are rich in water (94%); carbohydrates represent almost 3% while proteins are calculated around 1,2%; fibers 1% and fats, present in the seeds, only 0.2% (100g of tomatoes correspond to 17kcal). Tomatoes contain discrete amounts of vitamins B, ascorbic acid (vitamin C), vitamin D, and vitamin E, which provide antioxidant and vitaminizing properties to the tomato. Mineral components (iron, zinc, phosphorus and calcium associated with citrates, tartrates and nitrates) act in synergy ensuring remineralizing and anti-radical properties. The organic acids content such as malic acid, citric acid, succinic acid and glutamic acid are useful for promoting digestion (1).

Carotenoids and lycopene

The presence of pigments determines the colour of the tomato which, in phase of maturation, changes from green to ruby red. Lycopene is the main cause of the red colour of mature tomatoes. All natural foods with a rich pink to red colour generally contain lycopene. A lot of foods contain lycopene such as guava, watermelon, papaya and pink grapefruit but tomatoes are the biggest source. The content of lycopene in tomato berries depends on the variety and grade of ripeness, ripe tomatoes contain from 30 to 100mg of lycopene per kilo of fresh product (2).

Chemistry and biochemistry of lycopene

From the chemical point of view, Lycopene is a carotenoid, an acyclic isomer of β-carotene. It is an unsaturated hydrocarbon containing 11 conjugated double bonds and 2 unconjugated bonds (fig. 1). Double bonds can undergo isomerization cis-trans (2) The isomerization can be induced by light, thermal energy and chemical reactions. In nature Lycopene is found in the trans isomeric form which is the most stable (3-4). Lycopene is not synthesized by the body, but it comes from diet. Lycopene, from the consumption of fresh tomato or tomato juice, has a low bioavailability; tomato puree and paste are characterized by a greater bioavailability, this as a direct consequence of the processing that involves the trituration of the tissues and some heat treatments that increase the ratio between the cis and trans isomers (5-6-7). Bioavailability is strongly influenced by several factors including the isomeric conformation (*cis* isomers are more bioavailable than *trans* isomers), physical state (level of crystallization and size of lycopene crystals) and concomitant intake of dietary lipids. The lipids promote the solubilization of lycopene during digestion, its absorption in the intestinal mucosa (dissolved in chylomicrons) and transport to the tissues through the bloodstream. Differently from β-carotene, once taken by the body it is not converted to vitamin A and performs its charitable activities with completely different mechanisms. In both plasma and tissues (liver, testicles, adrenal glands, prostate and skin), Lycopene is present as cis isomer; in some (prostate and testicles), cis isomers account for more than 80% of the lycopene present. Due to the high number of dieni, lycopene is one of the most powerful natural antioxidants (8). Among natural carotenoids, in vitro this compound showed the highest scavenger against free radicals and it is able to inactivate a singlet-oxygen-quenching ability twice as high as that of β-carotene and 10 times higher than α-tocopherol (9). In vivo tomato and its derivatives have shown that reduce DNA damage, lower the susceptibility of the oxidative stress of lymphocytes and oxidation of the LDL or peroxidation of lipids (10-11). Besides lycopene for its antioxidant activity, can be used for the prevention or treatment of some neoplasms: induces apoptosis, increases the gap junctions, inhibits cell proliferation, interaction with growth factors and the sex hormones, induction of cellular

differentiation, immunomodulation, induction of phase II enzymes, anti-inflammatory action, antioxidant action, photoprotection and anti-angiogenesis. Various researchers have shown preventive and therapeutic role of lycopene in prostatic pathology, in cardiovascular diseases, in osteoporosis, in lung and breast tumors (2).

Skin and skin aging

This study will show especially the antioxidant and antiaging activity of lycopene. It is known that feeding and taking specific supplements can positively influence numerous structural and physiological features of the skin such as density, consistency, colour, hydration etc (12). The mechanism of skin aging includes intrinsic aging (chronological aging of the skin) and extrinsic aging (influenced by physical and chemical factors). Collagen provides the solidity of the dermis due to the interlocking of the collagen packed against one other in all directions. They are constituted of fibrils that are sealed together, thus forming more than ten types of different structures. The collagen fibers contribute to the elasticity and tonicity of the skin and/or the mucosae, they are continually renewed but this renewed decreases with age, leading to thinning of the dermis. This thinning may also be due to pathological causes, for example hypersecretion of corticoid hormones, some pathologies or also vitamin deficiencies (vitamin C in the case of scurvy). It is also assumed that extrinsic factors such as ultraviolet radiation, tobacco or certain treatments (glucocorticoids, vitamin D and derivatives for example) have also an effect on the skin and on its collagen level. Various factors lead to degradation of collagen, with all the consequences that can be imagined on the structure and/or firmness of the skin and/or of the mucosae (13).

Skin aging can be induced by oxidative stress, telomeres loss, mutations in mitochondrial DNA (mtDNA), hormonal changes and main reason of facial aging is due to exposure to solar radiation and ultraviolet rays (UVA and UVB). Intracellular and extracellular oxidative stress by reactive oxygen species (ROS) exacerbate skin aging, which is characterized by wrinkles and atypical pigmentation. Because ultraviolet (UV) enhances ROS generation in cells, skin aging is usually related to UV exposure. Nowadays, there are many cosmetic procedures and products which can effectively reduce the aging of skin and treat the wrinkles (14). The treatment of shallow-to-deep wrinkle usually begins with topical therapy. A variety of systemic and topical therapies are available for wrinkle treatment, and one of the most effective agents are antioxidants. They decrease ROS by direct scavenging, decreasing the amount of oxidation in and around our cell, prevention of ROS attaining their biological targets, limiting the spread of oxidants such as the one that occurs during lipid peroxidation, and stopping oxidative stress, thereby preventing the aging phenomenon(15). Lycopene's powerful antioxidant action and ability to defend the skin against UV radiation are due, largely, to its unique molecular design, which is responsible for lycopene's red appearance and its ability to block UV light. Sun protection of lycopene is only equivalent to approximately SPF-3 which is not adapt for sun protection by itself, but topically-applied lycopene has been shown to be able to defend against the harmful effects to UVB radiation (16). It was found that topical application of Lycopene suppressed the typical UVB-induced activity of an enzyme called ornithine decarboxylase, an important initiating and rate-controlling factor involved in stabilizing DNA structure in the nucleus of the skin cells as well as maintaining the DNA double-strand break repair pathway. What interests us above all is to highlight that lycopene is able to offer significant protection to the cellular DNA and thus negated the need for the body to activate its internal DNA repair pathway (16-17). UVB radiation also reduce an important substance in the skin known as PCNA (proliferation cell nuclear antigen), which is vital for DNA synthesis and cell repair. The topical application of lycopene was found to reverse the reduction of PCNA caused by UVB exposure to a significant degree. Then, it was shown that lycopene might also protect the skin through its ability to reduce inflammation, encourage cell renewal, and inhibit normal DNA damage following UVB injury which means it may help reduce the risk of wrinkles and protect skin against free radical damage (18).

Lycopene extraction

The lycopene used for the production of dietary supplements or other preparations can be produced by synthesis (synthetic lycopene), extracted from the plants (natural lycopene) or extracted from

plants grown using organic methods (biological lycopene). Synthetic lycopene is produced from synthetic raw materials dissolved in organic solvents. The process used (Witting Process) is very long and complex and leads to the formation of intermediates and raw lycopene crystals that are then purified by filtration and recrystallization. The lycopene crystals are large and the concentration of lycopene is 90/95% in weight but easily degrades to has a low bioavailability. As the size of the crystals decreases so the low bioavailability of lycopene increases. At the same high concentrations reducing the size of crystals by 5 µm to 0.5 µm bioavailability increases by 30%. The disadvantage of synthetic lycopene is that it can contain residues of organic solvents and other potentially toxic impurities. The extraction of lycopene from mature tomato berries can be carried out through a traditional process that uses organic solvents toxic to human health and the environment (natural lycopene) or through a process that uses carbon dioxide supercritical as the only extractive solvent (biological lycopene). Natural lycopene is extracted from fresh tomato or processing waste from the tomato industry through the use of organic chemical solvents (chloroform, hexane) from which it is separated by crystallization. Extraction is not selective as inside in addition to lycopene there are other lipophile substances that precipitate in crystals as impurities. These substances are not toxic and act synergistically by enhancing the antioxidant activity of the extract and the crystals that are formed are smaller and therefore the bioavailability is better. These crystals can be purified for recrystallization but this causes substances that work in synergy with lycopene to be lost. Organic lycopene is obtained through carbon dioxide extraction under supercritical conditions from a lyophilized tomato matrix prepared from mature berries grown using biological methods. The absence of organic solvents exclude contamination so the product is 100% completely free of toxic substance. Biological lycopene contains other carotenoids which contribute synergistically to the effects of lycopene. These supports are also found in higher quantities and conserve their biochemical characteristics so the biological lycopene has higher antioxidant activity. It is not found in the form of crystals but as an oversaturated solution of lycopene in vegetable oil rich in unsaturated fatty acids (oleoresin) (2-8). The aim of this article is to demonstrate the anti-aging activity of lycopene, after topical or oral administration on women.

Methods

In the first study different O/W emulsions with 5% w/w tomato powder were prepared and stability tests were performed. Lycopene was analyzed by paper chromatography and UV spectrum and after it was measured in tomato powder. To develop a stable emulsion, some formulations using experimental design were prepared. Then quality control test of formulated lotion was carried out. Therefore anti-aging activity was evaluated by Visioface about wrinkles decrease. In the second study, instead, the efficacy of dietary supplement A was evaluated by a dermatologist by means of various photographic atlases and by instrumental measurements (Torquemeter R and image analysis based on skin prints).

Results

First study

The first research (14) had aimed to evaluate the physical stability and anti-wrinkle activity in a lotion of tomato powder (consist of lycopene).

The research measured the anti-wrinkle activity with Visioface devices, and antioxidant activity with densyl chloride. Lotions are emulsion of oleaginous substances and water, and spread more easily over skin than ointments. Oil-in-water (O/W) types of lotions are easily water-washable. Advantages of bringing the drugs in lotion bases are: lotion hydration prevents development of shallow wrinkles induced by dehydration oh skin. Moreover, moisture accumulates between the skin and the lotion layer that causes hydration of the stratum corneum. Hydration of stratum corneum allows "opening up" of intra- and inter-cellular channels and pathways for easier passage of drug molecules. Additionally, the moisture layer provides a medium for dissolution of the drug that is otherwise dispersed as fine particles in the lotion base. The first clinical test was the skin irritation test.

The formulation was applied over the arms of 10 women. The test sites were observed for erythema and oedema for 48 hours after application. The prepared formulation did not show any erythema or oedema; this indicates that the prepared formulation containing 5% tomato powder was not-irritant on skin (20). The second test was the

application of the formulation contents 25mg lycopene in 100g lotion to demonstrate the anti-aging action of lycopene.

20 healthy women with more than 30 years, divided into 2 groups, with wrinkling and pigmentation as its main indications. was recluted.

To the first group of ten women was applied the lotion base with tomato powder and compared them with other group of 10 women as control that used placebo (lotion base without tomato powder). These two groups were compared via charts get from Visioface software. To use the Visioface it is necessary to fix the head of volunteers in front of the apparatus, on the marked place, and then to select the capture from menu, then it's possible toanalyse the photo. This software is able to measure the pixels of volume, area and depth of the wrinkles.

The wrinkles measurements via charts were obtained from Visioface in 10 weeks and the data collected at days 7, 14, 21, 28, 35, 42, 56, 63 and 70. The digits produced by Visioface in week 1 and week 6 are presented in table 1. The most efficacy about wrinkle decrease was in day 42 (week 6) and after that was no more advance in wrinkle reduction. Wrinkle chart in day 0 and day 42:

Px^3= volume of wrinkles at pixel

Px^2=area of wrinkles at pixel

Px=depth of wrinkles at pixel

The effects of applied lotion were significant after 6 weeks and were measured by Visioface devices. Based on the experimental results, the average decrease in depth, area and volume of wrinkles were 26,4%, 24,3%, 38.1% respectively, which were improved compared to control group. These effects were observable in all of the cases receiving treatment with the tomato lotion formulation (Table 1).

Second study

The second test (13) relates more particularly to the prevention and/or treatment, by the oral and/or parenteral route, of skin conditions associated with a deterioration of the viscoelastic or biomechanical properties of the skin. It aims more particularly to maintain and/or restore the biomechanical properties of the skin. More precisely, the inventors discovered that the administration, by the oral and/or parenteral route, of a combination of lycopene, vitamin C, vitamin E and at least one polyphenol compound derived from pine bark, displays a beneficial activity on keratinous materials and in particular on the constituents of the dermis, and makes it possible to combat a deterioration of the biomechanical properties of a keratinous material, in particular of a connective tissue and more particularly of the skin. Topical treatments for combating the cutaneous signs notably associated with aging are known. However, the topical active ingredients recommended do not always have an action at the level of the dermis, owing to poor penetration through the skin. Moreover, topical products act, by definition, locally on the zones to be treated, and they may be distributed nonuniformly on said zones, and require careful, repeated applications. They may in some cases cause cutaneous side effects, or discomfort. In contrast, the oral route has the advantage of acting globally on all of the skin and in its deep layers (dermis, hypodermis), following a method of administration that is quick and not very restricting. In fact, the metabolites and other active nutrients are in particular distributed within the dermal matrix via the blood circulation. The oral route or administration by patch also after the advantage of a method of administration that is quick and not very restricting. The lycopene can be aqueous suspension. For this, it is possible to use water-dispersible forms, cold or hot or an oleoresin extracts containing from 6% to 10% of pure lycopene, for example a primary composition comprising lycopene and a whey protein. It has the advantage of increasing the bioavailability of the lycopene and/or of being easily formulated in dietary supplements (as sachet, capsule, tablet, coated tablet, soft capsule, etc.). In the study is administered supplement A (tablets containing lycopene 100mg/cp, vitamin C 28.9 mg/cp, extract of maritime pure bark 13.3 mg/cp and vitamin E 4.9mg/cp – see table 2) as film-coated tablets with a posology of 2 tablets/day in postmenopausal women who have loss of skin firmness. Objective of clinical test is the investigation of the effect of taking dietary supplement A in the deterioration of the biomechanical proprieties of the skin and of the micro relief of the skin. Single-centre, double blind study on 2 groups, one of which is a placebo group. Seventy-two healthy female volunteers, aged from 40 to 65 years and meeting the criteria for inclusion

and non- inclusion participated in this study, with a total duration of 6 months. 49 participants are given supplement A and 23 the placebo. The efficacy of dietary supplement A was evaluated: by a dermatologist by means of various photographic atlases; by a dermatologist according to a 6-point clinical score from 0 to 5. The more the state of the parameter is regarded as good, the closer the score is to 5 and vice versa; by instrumental measurements (Torquemeter® and image analysis based on skin prints); by a self-assessment questionnaire completed by the volunteers.

In the group who had taken supplement A, the depth of crow's-feet wrinkles is statistically reduced starting from 12 weeks (T12/T0, p=0.003). Taking dietary supplement A also leads to a statistically significant decrease in wrinkles at the corner of the lips starting from 12 weeks (T12/T0, p=0.048), as well as of the bags under the eyes 24 weeks (T24/T0, p=0.034). These parameters show no change in the placebo group (tables 3-4).

Conclusion

In both studies either topical and systemic administration of lycopene resulted in significant reduction of wrinkles. The first study shows that tomato powder formulated in lotion base significantly decreased wrinkles in test group. This formulation is compatible with skin and caused no hypersensitivity reaction in tested human model. The second one shows that dietary supplement A makes it possible to correct the undesirable signs, notably cutaneous, associated with loss of biochemical characteristics in particular of the skin, improving the topography as well as the relief of the skin. Dietary supplement A aids in restructuring the skin on the inside for a skin that is firmer and for tangible results at the surface.

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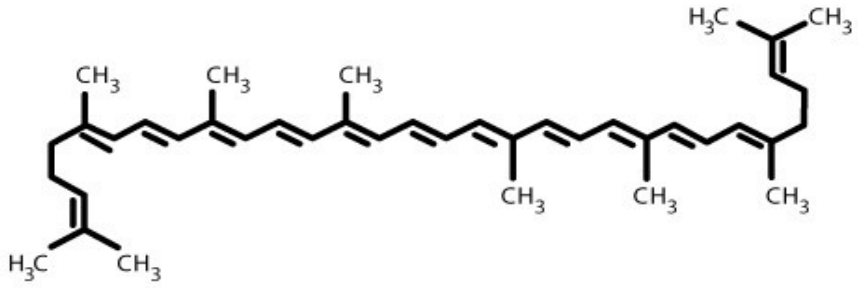

Figure 1*-Chemical structure of Lycopene (2)*

Case 1	**Week 1**	**Week 6**
Volume	150.451	Px^3= 100.145
Area	10.199	Px^2= 8.45
Depth	18.019	15.035
Case 2	**Week 1**	**Week 6**
Volume	140.541	120.654
Area	9.205	7.456
Depth	15.349	12.023
Case 3	**Week 1**	**Week 6**
Volume	178.45	90.81
Area	5.65	3.58
Depth	16.523	15.363

Table 1*-The digits produced by Visiofase in week 1 and week 6 (14)*

	Common name of ingredient/excipient	Composition (mg/cp)
NUTRITIONAL INGREDIENTS	Lactolycopene 2%	100.00
	Vitamin C 90%	28.9
	Extract of maritime pine Bark	13.3
	Vitamin E	4.9

Table 2-Composition of Dietary supplement A (13)

Table 3*-Variation of cutaneous sign – Dietary supplement A (13)*

Variation of cutaneous signs - Dietary supplement A						
PARAMETERS	To (mean ± SD)	T12 (mean ± SD)	T12/To Turkey test for multiple comparisons	T24 (mean ± SD)	T24/To Turkey test for multiple comparisons	Analysis of variance
Depth of crow's – feet wrinkles	3.3 ± 1.2	2.6 ± 0.9	Improvement P=0.003	2.8 ± 1.6	Improvement P=0.045	Improvement P=0.004
Bags under eyes	2.4 ± 1.3	2.0 ± 1.2	NS	1.8 ± 1.1	Improvement P=0.034	Improvement P=0.012
Wrinkles on forehead	2.9 ± 1.1	1.9 ± 1.2	Improvement P=0.048	2.0 ± 1.3	NS (rend. P=0.055)	Improvement P=0.027
Wrinkles at corner of lips	2.5 ± 1.5	1.9 ± 1.6		1.7 ± 1.8		

Variation of cutaneous signs - Placebo						
PARAMETERS	To (mean ± SD)	T12 (mean ± SD)	T12/To Turkey test for multiple comparisons	T24 (mean ± SD)	T24/To Turkey test for multiple comparisons	Analysis of variance
Depth of crow's – feet wrinkles	2.4 ± 1.1	2.0 ± 0.9	Improvement P=0.003	1.8 ± 1.0	Improvement P=0.045	Improvement P=0.004
Bags under eyes	2.7 ± 1.2	2.1 ± 1.1	NS	1.8 ± 1.2	Improvement P=0.034	Improvement P=0.012
Wrinkles on forehead	2.9 ± 1.1	1.9 ± 1.2	Improvement P=0.048	2.0 ± 1.3	NS (rend. P=0.055)	Improvement P=0.027
Wrinkles at corner of lips	2.5 ± 1.5	1.9 ± 1.6		1.7 ± 1.8		

Table 4*-Variation of cutaneous signs – Placebo (13)*

SD standard variation
NS not statistically significant